Electronic supplementary information to

Liquid chromatography mass spectrometry based characterization of epitope configurations

Maren Christin Stillesby Levernæs[1], Arelí Urtubia Moe[1], Sigurd Leinæs Bøe[2], Elisabeth Paus[2], Léon Reubsaet[1], Trine Grønhaug Halvorsen*[1]

[1]Department of Pharmacy, University of Oslo, Norway
[2]Department of Medical Biochemistry, Norwegian Radium Hospital, Oslo University Hospital, Norway

*Corresponding author:

Trine Grønhaug Halvorsen

Address: Department of Pharmacy, PO Box 1068 Blindern, 0316 Oslo, Norway

Phone: +47 2285 5735

E-mail: trinegha@farmasi.uio.no

Supplementary Results and Discussion

S.1 Choice of sample matrix

To minimize falsely elevated signals due to non-specific binding extraction of ProGRP and hCG from ABC solution and serum was performed with a non-ProGRP antibody and non-hCG antibody, respectively (i.e. negative control experiments). Not surprisingly, the non-specific binding was highest when ABC solution was used as matrix (≤ 12.2 %, Table S1). When using serum as matrix the non-specific binding was reduced to ≤ 2.9 %, which was found to be acceptable (Table S1). This suggests using serum as sample matrix.

Table S1. Evaluation of non-specific binding using different sample matrices

	Non-specific binding[1] in	
	Serum	ABC solution
hCG		
GVNPVVSYAVALSCQCALCR	0.84 %	0.23 %
VLQGVLPALPQVVCNYR	0.49 %	0.19 %
ProGRP		
NLLGLIEAK	2.9 %	12.2 %
ALGNQQPSWDSEDSSNFK	1.5 %	7.4 %

[1]Estimated as %-binding to non-analyte antibody compared to when using analyte antibody.

However, serum from healthy individuals contains the model analytes at their reference levels. To estimate the influence of their presence, extraction of blank serum samples was performed with all mAbs investigated in this study. The signal intensity of the signature peptides in these samples was compared with the signal intensity after preparation of a spiked sample. Levels of endogenous hCG were detected, but as this signal account for less than 1 % of the signal generated from a spiked sample, these levels were considered to be negligible. Endogenous levels of the other proteins used (ProGRP and Tg) where not detected using the current analytical system (nanoLC-LTQ-Orbitrap).

S.2 Verification of experimental set-up

In the designed experimental set-up the target proteins were treated with denaturant and/or reduction and alkylation agent prior to immunocapture. The samples were diluted before the antibodies were added but still the immunocapture was performed in the presence of certain amounts of urea and/or DTT and IAA in the sample that may compromise the performance of the antibody. The potential impact of this was investigated using the following procedure: Antibody coated magnetic beads (mAb E146 or E27) were incubated with 0.8 M urea and/or 1 mM DTT and 4 mM IAA for 1 hour

at room temperature (levels present during immunocapture in the test procedure). After incubation, the antibody-coated beads were washed and used to extract intact ProGRP (250 ng/mL using E146) or hCG (5 µg/mL using E27) from spiked samples followed by on-beads trypsin digestion overnight. The signal intensities of the signature peptides generated after digestion were compared to those generated from an extraction of intact ProGRP or hCG by antibody coated beads not exposed to urea, DTT and IAA prior to extraction (control).

One of the three signature peptides investigated for ProGRP showed a significant decrease in signal intensity ($P < 0.05$) when the antibody had been exposed to urea, DTT and IAA compared to the control (Figure S1A). However, as there was no significant difference in the signal intensity of the two other signature peptides investigated for the same protein ($P > 0.05$), nor for any of the signature peptides monitored for hCG (Figure S1B), the antibodies extraction efficiency was considered to be unaffected by the urea, DTT and IAA concentrations in the sample. The observed difference in signal intensity for this one peptide is most likely due to differences in digestion efficiency.

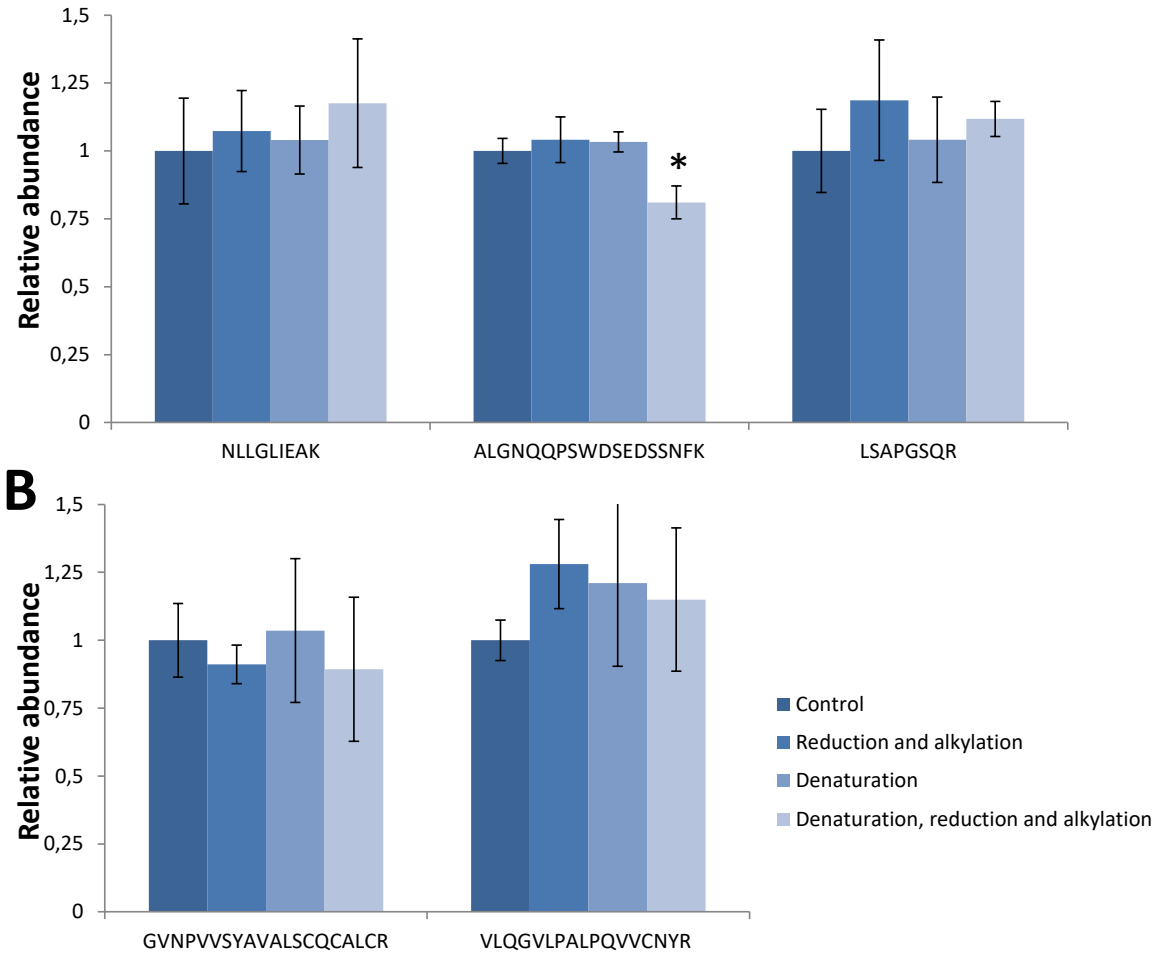

Figure S1. Verification of mAbs ability to extract target protein in presence of pre-treatment reagents.

Relative abundance of selected signature peptides of ProGRP (A) and hCG (B) after pre-treatment of the protein standard with either reduction and alkylation, denaturation, or denaturation, reduction and alkylation prior to immunoextraction. *Significantly different from the control (pre-treatment reagents not present) (P-value < 0.05). Error bars represent standard deviation.